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Small RNAs Shed Some Light

Small regulatory RNAs can act by pairing with their target messages, targeting themselves and the mRNA for degradation; Lenz et al. (this issue of *Cell*) now report that multiple small RNAs are essential regulators of the quorum-sensing systems of *Vibrio* species, including the regulation of virulence in *V. cholerae*.

Small noncoding regulatory RNAs have become the recent rage in both prokaryotes and eukaryotes. Lurking in eukaryotic introns and bacterial intergenic regions are short RNA transcripts that can negatively and positively regulate gene expression, usually by perturbing mRNA use and stability. In eukaryotes, microRNAs are essential regulators of development in plants and animals, and double-stranded RNAs both signal defense from viral invaders and localize chromosome silencing around centromeres (reviewed in Bartel [2004]). In prokaryotes, small RNAs regulate the transition to stationary phase, iron homeostasis, and polarity in some operons (reviewed in Gottesman [2004]; Storz et al. [2004]). A major class of these small RNAs (sRNAs) act by binding to the RNA chaperone Hfq, a member of the Sm family of RNA binding proteins, followed by pairing to specific target mRNAs. The consequence of pairing can be stimulation or inhibition of translation or stimulation or inhibition of mRNA decay (reviewed in Gottesman [2004]; Storz et al. [2004]).

In the paper by Bassler and coworkers in the current issue of *Cell* (Lenz et al., 2004), small RNAs make their appearance at the central regulatory step in the quorum-sensing cascade that regulates bioluminescence in the marine bacteria *V. harveyi* and virulence in *V. cholerae*. Quorum sensing is the process bacterial cells use to sense whether they are alone or in a crowd; they send out small molecules into the medium and then measure how many of the small molecules are present in the medium as a measure of how many other bacteria are also excreting the small molecule signaler (reviewed in Miller and Bassler [2001]). In *Vibrio harveyi*, the output of the quorum-sensing pathway is expression of genes for luciferase, encoded by *lux*; the bacteria, which lives in the light organ of a fish, makes light at high density (Miller and Bassler, 2001). The production of light (and therefore expression of the *lux* genes) can be used as a simple reporter in either its natural host or in *V. cholerae*. In both organisms, it was known that different quorum-sensing pathways converged on a single positive regulator, LuxO. LuxO is a member of the family of transcriptional activators that is known to interact with and activate a special class of promoters, those recognized by bacterial RNA polymerase containing the promoter specificity factor sigma 54. However, elegant genetic analyses had shown that this positive regulator resulted in the *negative* regulation of a downstream regulator, the gene immediately upstream of *lux* in the signaling cascade (LuxR in *V. harveyi* and HapR in *V. cholerae*) (Lilley and Bassler, 2000) (Figure 1). These data suggested that LuxO was positively regulating a negative regulator, which in turn was regulating LuxR and HapR. Lenz et al. went on a hunt for this putative negative regulator, beginning with a classic genetic screen. Starting with a strain in which LuxO is always active and the *lux* genes are therefore always off (as in Figure 1A), mutations that could express light were identified. The resulting mutations were in the gene encoding the Hfq chaperone. Experiments over the last five years have shown that if Hfq is needed, small RNAs may be involved as well. If such sRNAs were the putative negative regulators, they should be made from LuxO-dependent, sigma 54-dependent promoters. Lenz et al. searched the *Vibrio* genomes for conserved small RNA-like sequences with sigma 54 promoters. In fact, not one but four such regions were found in *V. cholerae*, encoding four unlinked but homologous sRNAs named Qrr1-4 (quorum-sensing RNAs). Mutational inactivation of all four was necessary to create the predicted phenotype: total loss of the response of *V. cholerae* to quorum sensing. The *V. cholerae* small RNAs are made in response to LuxO signaling that mimics the low density growth condition, and expression of the sRNAs leads to rapid degradation of the mRNA for HapR (and LuxR in *V. harveyi*). Thus, positive regulation by LuxO and sigma 54 becomes negative regulation of HapR (Figure 1).

The Qrr RNAs solve a regulatory puzzle and clearly extend the important role of small regulatory RNAs in bacteria firmly into the realm of regulation of virulence. *Vibrio cholerae* virulence genes are only expressed when the small RNAs are made. Once made, the Qrr RNAs appear to resemble the *E. coli* RyhB RNA in function. RyhB, which is under the regulation of the Fur repressor, negatively regulates iron-containing proteins when iron is limiting. Negative regulation is accompanied by rapid RNaseE-dependent coupled degradation of both the target message and the small RNA itself (Massé et al., 2003). Lenz et al. find that HapR mRNA is destabilized when the small RNAs are present, and the amounts of the Qrr RNAs increase in the absence of the target gene, consistent with co-degradation of sRNA and target.

Why use small RNA regulators for quorum sensing, and why are there four apparently redundant RNAs? Small RNAs can be made quickly, and can be turned off quickly, as a result of their degradation. Their action can be epistatic to transcriptional signals for the same target genes, and multiple genes can be regulated by a single sRNA (Massé and Gottesman, 2002). Multiple related sRNAs may allow more subtle variations in regulation, both because they may be made under somewhat different conditions and because the differences in sequence will lead to pairing and degradation of mRNAs with different efficiencies. For the positive regulation of RpoS translation by small RNAs, a number of different small RNAs, each made under a different stress condi-

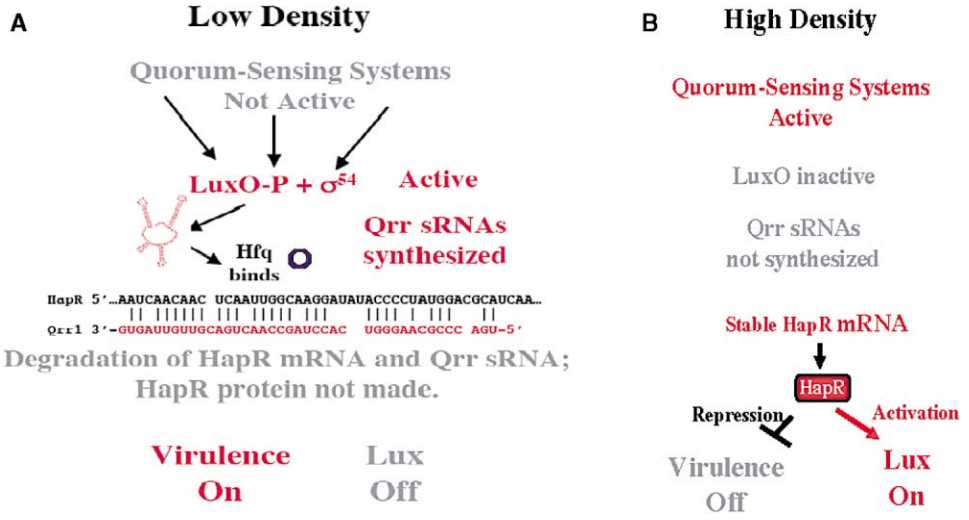

Figure 1. Low Density Signaling for Qrr RNA Synthesis

(A) In the absence of quorum sensing (low density), LuxO is phosphorylated. The phosphorylated form is a positive regulator of Qrr RNA synthesis; the Qrr RNAs negatively regulate HapR mRNA stability and translation.

(B) At high density, LuxO is not phosphorylated, and the Qrr RNAs are not made. See Lenz et al. (2004) for more details.

tion, act on the same region of the same target message (reviewed in Repoila et al. [2003]). In *Pseudomonas aeruginosa*, two redundant small RNAs with subtly different induction conditions regulate iron homeostasis (Wilderman et al., 2004). sRNAs with varying degrees of redundancy may turn out to be a common cell strategy for precise regulation.

The appearance of four small RNAs in the center of the signaling cascade for quorum sensing and virulence is undoubtedly a sign of things to come. In fact, a regulatory RNA, RNAIII, has been known for years to play a critical role in *S. aureus* virulence (reviewed in Johansson and Cossart [2003]). Watch for small RNAs in all your favorite regulatory circuits.

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Neddylating the Guardian: Mdm2 Catalyzed Conjugation of Nedd8 to p53

The tumor suppressor and transcriptional regulator p53 is perhaps one of the most regulated proteins in the cell nucleus and is acted upon by a variety of protein kinases, acetylases, ubiqutin ligases and hydrolases, and SUMO-conjugating enzymes. Now new work suggests a role for an additional modification—neddylation—in negative regulation of p53 transcriptional activity.

The tumor suppressor p53 is a central component in the signal transduction pathway that responds to cellular and genotoxic stress. p53, through its transcription factor activity, induces the expression of genes involved in DNA repair, cell cycle arrest, and apoptosis. The key position that p53 takes in the stress pathway dictates that its activity be highly regulated, and multiple mechanisms, including phosphorylation, acetylation, and ubiquitination-mediated degradation, conspire to regulate its activity (Yang et al., 2004). To the casual observer, the status of our understanding of p53 and its regulation is likely to present a paradox. On one hand, p53 is already one of the most intensively studied proteins in